ISSN 0840-8440

PROCEEDINGS

TECHNOLOGY TRANSFER CONFERENCE 1988

November 28 and 29, 1988

Royal York Hotel

Toronto, Ontario

SESSION D

ANALYTICAL METHODS

Sponsored by

Research and Technology Branch

Environment Ontario

Ontario, Canada

D4

PROCEDURES FOR THE 2,3,7,8-SUBSTITUTED ANALYSIS OF PCDD & PCDF AND OTHER TARGET COMPOUNDS IN ENVIRONMENTAL SAMPLES

F. W. KARASEK*, T. S. THOMPSON AND K. P. NAIKWADI
DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF WATERLOO,
WATERLOO, ONTARIO N2L 3G1

PROCEDURES FOR THE 2,3,7,8-SUBSTITUTED ANALYSIS OF PCDD & PCDF AND OTHER TARGET COMPOUNDS IN ENVIRONMENTAL SAMPLES

Introduction:

Chlorinated pollutants have for years been a major concern because of their toxicological properties and widespread existence in the environment. Over the last decade two classes of organic compounds known as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have received considerable attention because of their extraordinary toxicities. These compounds have become virtually ubiquitous in the environment having been identified as contaminants in incinerator flyash, sediment, water, human milk, and biological tissue.

The basic structures of the dioxin and furan molecules are shown in figure 1 along with the eight sites which may be substituted with a chlorine atom. The spatial arrangement of one to eight chlorine atoms yields a total of 75 different PCDD isomers while the less symmmetrical furan molecule gives rise to 135 isomers. Figure 1 also shows the numbering scheme employed in the PCDD/PCDF nomenclature.

Although PCDDs and PCDFs have many similar chemical and physical properties, the toxic behaviour of individual isomrs may vary dramatically. Table I lists the LD_{50} values for several PCDD isomers [1]. The most

toxic dioxin and furan isomers are the 2,3,7,8-tetrachlorinated compounds, 2,3,7,8-TCDD and 2,3,7,8-TCDF respectively. There are however several other PCDD and PCDF isomers which have been found to be highly toxic. This group of toxic isomers consists of the PCDDs and PCDFs which have four to six chlorine atoms and all four lateral sites (positions 2, 3, 7, and 8 in figure 1) occupied by chlorines [2]. Thus there are twelve compounds, five PCDDs and seven PCDFs, which are highly toxic. These compounds are listed in table II.

Most PCDD and PCDF analyses have previously involved the determination of total congener levels for the tetra- through octachlorinated species and/or the isomer specific determination of 2,3,7,8-TCDD and 2,3,7,8-TCDF. The high toxicity of the ten other 2,3,7,8-substituted dioxins and furans makes it desirable to perform isomer specific determinations for these compounds.

The isomer specific determination fo PCDDs and PCDFs is hindered by the fact that no single GC column is capable of resolving all the individual isomers. Buser and Rappe prepared all of the tetra-, penta-, and hexachlorinated dioxin isomers and attempted to separate the five most toxic isomers from the remaining PCDDs [3]. Usihg a 55 metre Silar 10c glass capillary column, the five most toxic dioxins were reasonably well separated from the other isomers. The toxic 2,3,7,8-TCDD was only partially resolved from 1,4,7,8-TCDD and two of the toxic hexachlorinated dioxins, 1,2,3,6,7,8-H_6CDD and 1,2,3,4,7,8-H_6CDD were found to closely elute. While there appears to have been some success in the isomer specific analysis of the highly toxic dioxins, very little work has been reported on the isomer specific determination of furans.

In this study we have investigated the combined use of HPLC and GC separation techniques for the isomer specific determination of 2,3,7,8-substituted dioxins and furans.

Experimental:

Individual PCDD and PCDF standards were obtained from the Ontario Ministry of the Environment. All organic solvents were distilled in glass by the supplier (BDH Chemicals Inc., Toronto, Ontario) and were suitable for pesticide analysis. A flyash extract used to investigate the separation capabilities of the alumina normal phase HPLC (NP-HPLC) fractionation. This extract was obtained by Soxhlet extracting approximately 40 grams of municipal waste incinerator flyash (Commissioner Street, Toronto, Ontario) with 350 mL of benzene for 24 hours. The extract was fractionated on silica NP-HPLC to isolate the dioxins and furans from the bulk of the matrix prior to performing the alumina NP-HPLC fractionation.

HPLC fractionations were accomplished using a Waters liquid chromatographic system which consisted of three model 510 pumps and a model 481 variable wavelength ultraviolet (UV) detector. The HPLC is controlled by a Waters 820 chromatography workstation consisting of an NEC APCIV personal computer and monochrome monitor. The workstation is linked to the pumps and detector by a system interface module.

Sample was introduced into the HPLC via a loop injector which consisted of a Rheodyne six-port valve with a 20 microlitre sample loop. The organic solutions are loaded into the loop through a needle port using a flat-tipped 100 microlitre Hamilton syringe.

The sample fractionations were achieved using an analytical scale alumina column (25 cm x 0.4 cm i.d.). This column was prepared in our laboratory using a Shandon packing pump (Shandon Southern Instruments Inc., Wewickley, Pennsylvania). A slurry of 5 micron alumina particulates (Rayonics Scientific Inc., Downsview, Ontario) in methanol was packed under a pressure of approximately 9000 psi.

The gradient elution program used for the separation is given in table III.

The HPLC effluent was collected using a Gilson model 201 fraction collector. The fraction collector was set up to collect the effluent at discreet intervals as established through the injection of standard

solutions of PCDDs and PCDFs under the identical gradient elution program. The fraction collection times are listed in table III.

All gas chromatography-mass spectrometry analyses were performed using a Hewlett-Packard HP5987A GC-MS. The HP5880A gas chromatograph is linked to the quadrupole mass spectrometer by a direct capillary interface. This permits the end of the GC column to butt up against the mass spectrometer ion source. All of the GC effluent therefore enters the mass spectrometer. A cool on-column injector and 30 metre DB-5 fused silica capillary column (0.32 mm i.d.) were used for all GC-MS analyses. The GC-MS is controlled by an HP1000 data system which is also linked to various peripheral devices. For the selected ion monitoring determination of PCDDs and PCDFs, three ions were chosen for each congener group. These ions corresponded to the most intense peaks in the molecular ion cluster for the various congeners.

Results and Discussion:

Two standard solutions containing a total of 15 PCDD and PCDF isomers were injected on alumina NP-HPLC. The resulting chromatograms are shown in figure 2. By collecting fractions at the intervals listed in table III and subsequently analyzing by GC-MS, the elution behaviour of the dioxins and furans was determined.

Clearly the elution behaviour does not depend solely upon the degree of chlorination and therefore it may be possible to exploit this selectivity. To investigate this possibility, a flyash extract believed to contain virtually all PCDD and PCDF isomers was fractionated on alumina NP-HPLC.

The flyash extract, which had been pre-cleaned using silica NP-HPLC, was injected on alumina NP-HPLC and fractionated using the conditions described in table III. The HPLC UV trace for the flyash extract along with the fraction collection intervals is shown in figure 3. Each of the collected fractions was subsequently concentrated to 20 microlitres and analyzed by GC-MS. Figures 4 through 10 show the reconstructed ion chromatograms for the dioxins and furans in the fractions as determined by selected ion monitoring. The highly toxic isomers have been labelled in their respective chromatograms.

As expected, 2,3,7,8-TCDF is collected in the fourth fraction. More importantly, the majority of the other TCDF isomers elute within the first three fractions. A single TCDF isomer elutes after 2,3,7,8-TCDF in the fifth fraction. The alumina NP-HPLC separation appears to be very effective in isolating the toxic 2,3,7,8-TCDF from the remaining TCDF isomers.

The majority of the total TCDD elutes within the first two fractions. 2,3,7,8-TCDD elutes between 16 and

18 minutes along with 1,2,3,4-TCDD and three other TCDD isomers. The 1,2,3,4-TCDD and 2,3,7,8-TCDD are not resolved by the DB-5 column however many other columns will resolve these two isomers.

Fraction 3 also contains several other highly toxic isomers as indicated in figure 6. Two of the toxic P_5CDFs, 1,2,3,7,8-P_5CDF and 2,3,4,7,8-P_CDF, are separated from the bulk of the remaining isomers which elute largely in fractions 1 and 2.

Conclusions:

The results obtained for the flyash extract indicate that the alumina column exhibits considerable selectivity. Many of the toxic isomers can be separated using a combination of HPLC and GC techniques. A further experiment involving fish tissue samples fortified with labelled dioxins and furans is currently underway and should yield more information regarding the applicability of the method to environmental samples.

References:

1. G. Choudhary in "Chlorinated Dioxins and Dibenzofurans in the Total Environment", G. Choudhary, L.H. Keith, and C. Rappe (ed.'s), Butterworth Publishers, Boston, Massachusetts, 1983, 416 pp.

2. H.R. Buser and C. Rappe; Anal. Chem., 56(3), 442-448, 1984.

3. H. R. Buser and C. Rappe; Anal. Chem., 52(14), 2257-2262, 1980.

TABLE I

Lethal Dosages of Selected Dioxins

DIOXIN ISOMER	*LETHAL DOSAGE* (LD_{50} ug/kg)* GUINEA PIGS	MICE
2,7-D_2CDD	> 2,000,000	> 8,000,000
2,8-D_2CDD	> 300,000	> 150,000
2,3,7-T_3CDD	29,444	> 3,000
2,3,7,8-T_4CDD	2	284
1,2,3,4-T_4CDD	> 1,000,000	
1,2,3,7,8-P_5CDD	3	338
1,2,4,7,8-P_5CDD	1,125	> 5,000
1,2,3,6,7,8-H_6CDD	70-100	1,250
1,2,3,7,8,9-H_6CDD	60-100	1,440
1,2,3,4,6,7,8-H_7CDD	180	

* taken from reference 1

TABLE II

Highly Toxic PCDDs and PCDFs

PCDDs:	2,3,7,8-TCDD
	1,2,3,7,8-P_5CDD
	1,2,3,6,7,8-H_6CDD
	1,2,3,7,8,9-H_6CDD
	1,2,3,4,7,8-H_6CDD
PCDFs:	2,3,7,8-TCDF
	1,2,3,7,8-P_5CDF
	2,3,4,7,8-P_5CDF
	1,2,3,4,7,8-H_6CDF
	1,2,3,6,7,8-H_6CDF
	1,2,3,7,8,9-H_6CDF
	2,3,4,6,7,8-H_6CDF

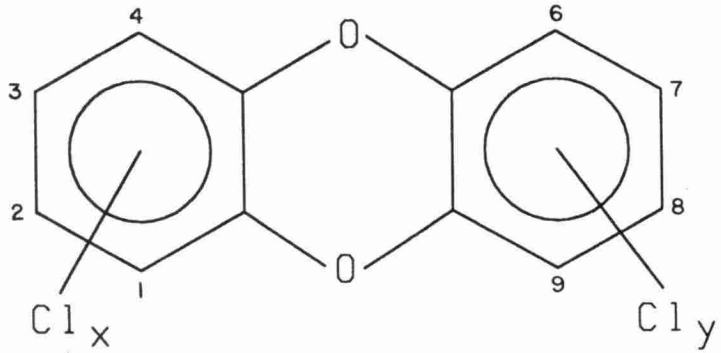
TABLE III

Alumina Normal Phase HPLC
Gradient Elution Program

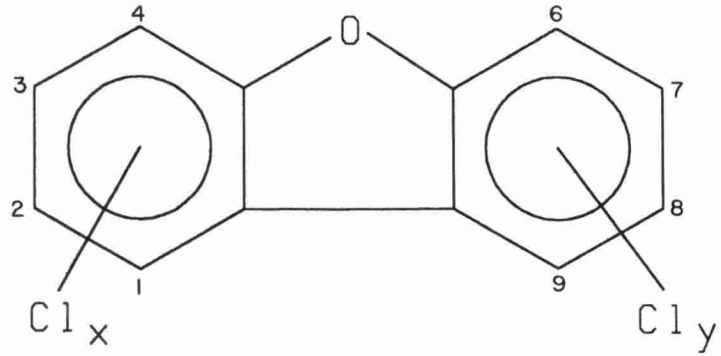
TIME (min)	FLOW RATE (mL/min)	MOBILE PHASE COMPOSITION % HEXANE	% DICHLORO-METHANE
0.0	2.0	100.0	0.0
10.0	2.0	100.0	0.0
11.0	2.0	95.0	5.0
30.0	2.0	95.0	5.0
35.0	2.0	0.0	100.0
55.0	2.0	0.0	100.0
60.0	2.0	100.0	0.0

Fraction Collection Times

FRACTION #	COLLECTION TIME
1	12 - 14
2	14 - 16
3	16 - 18
4	18 - 20
5	20 - 24
6	24 - 26
7	26 - 30

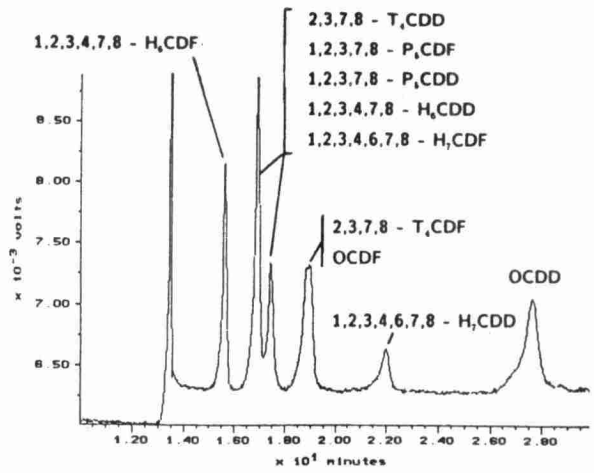


PCDDs



PCDFs

FIGURE 1: Structure of PCDDs and PCDFs



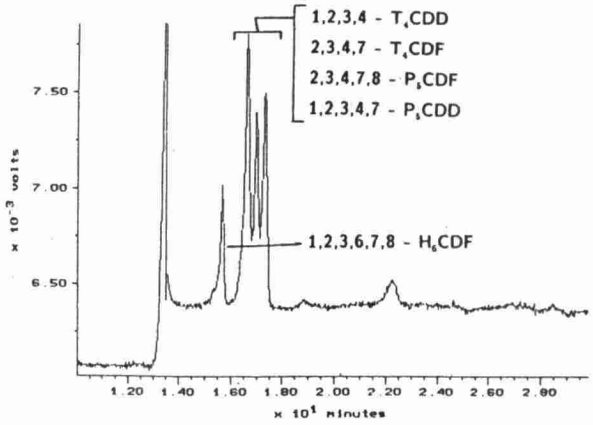


FIGURE 2: Alumina NP-HPLC of PCDDs and PCDFs

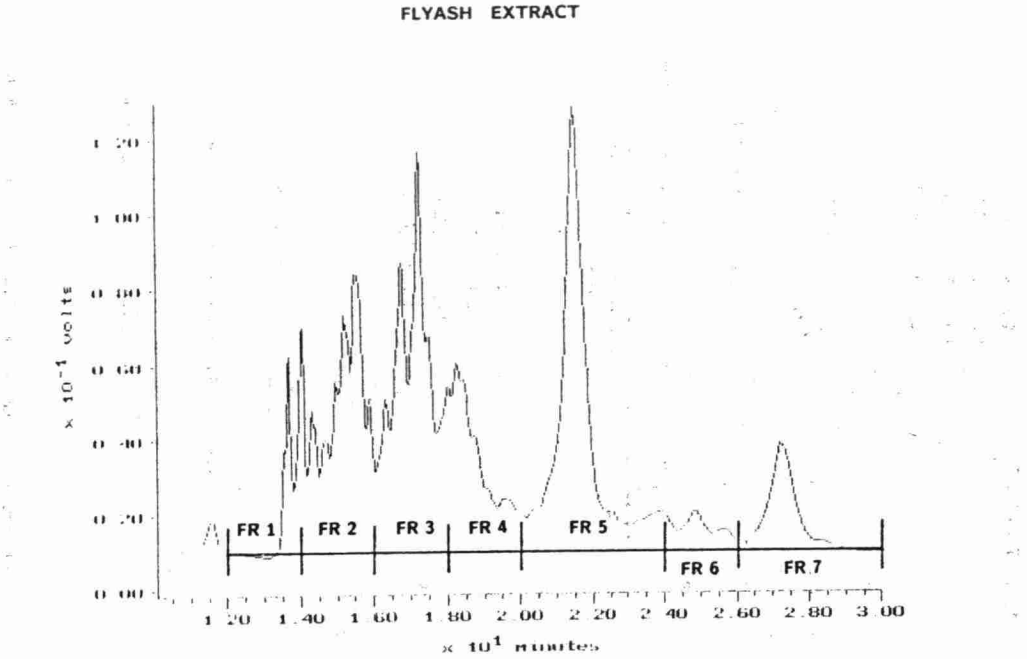


FIGURE 3: NP-HPLC fractionation of flyash extract.

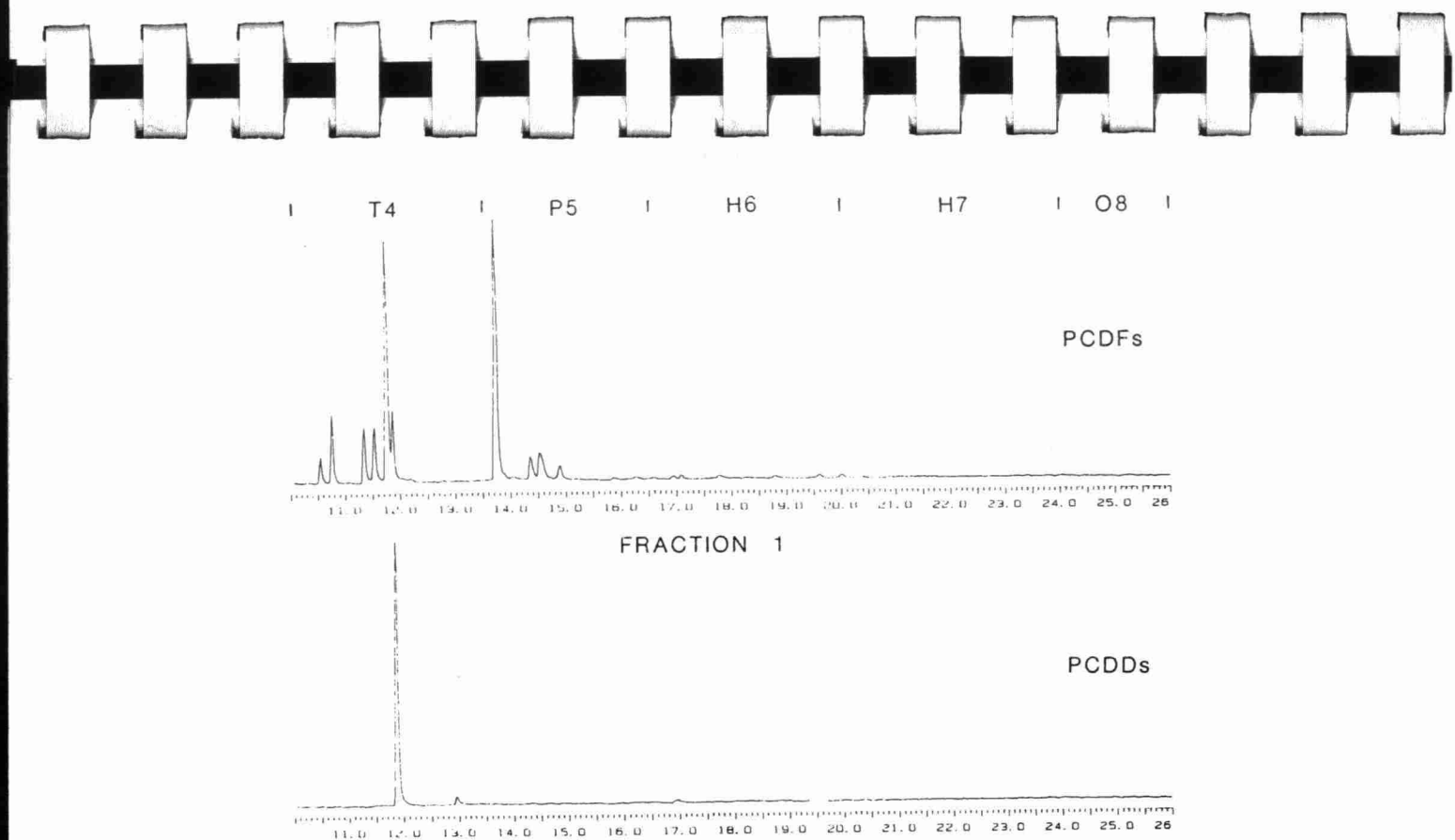


FIGURE 4: RIC of flyash fraction #1.

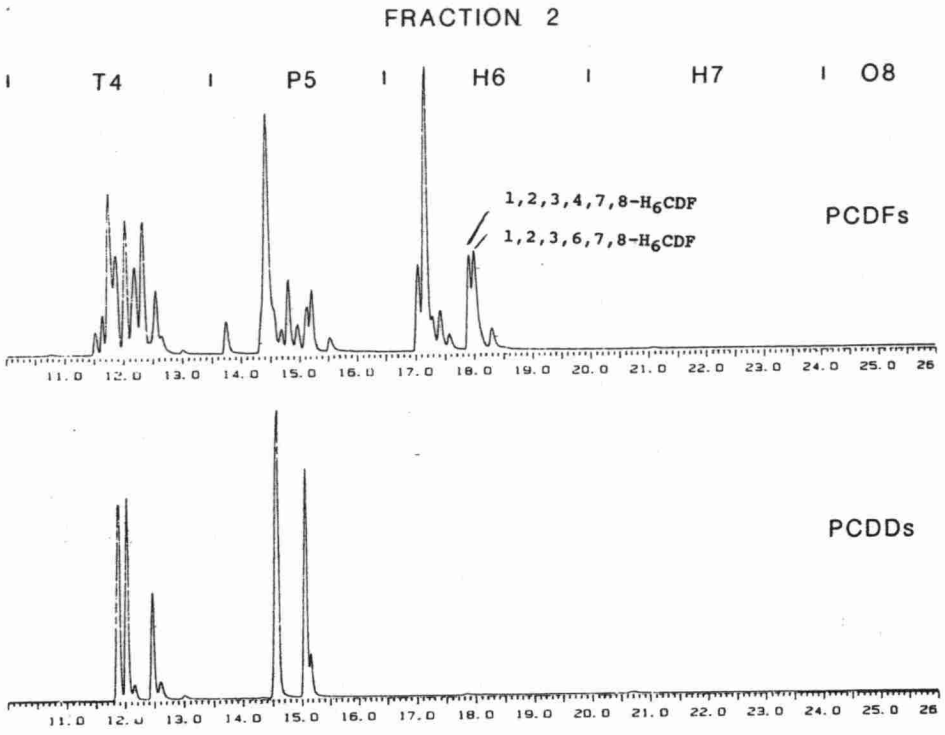


FIGURE 5: RIC of flyash fraction #2.

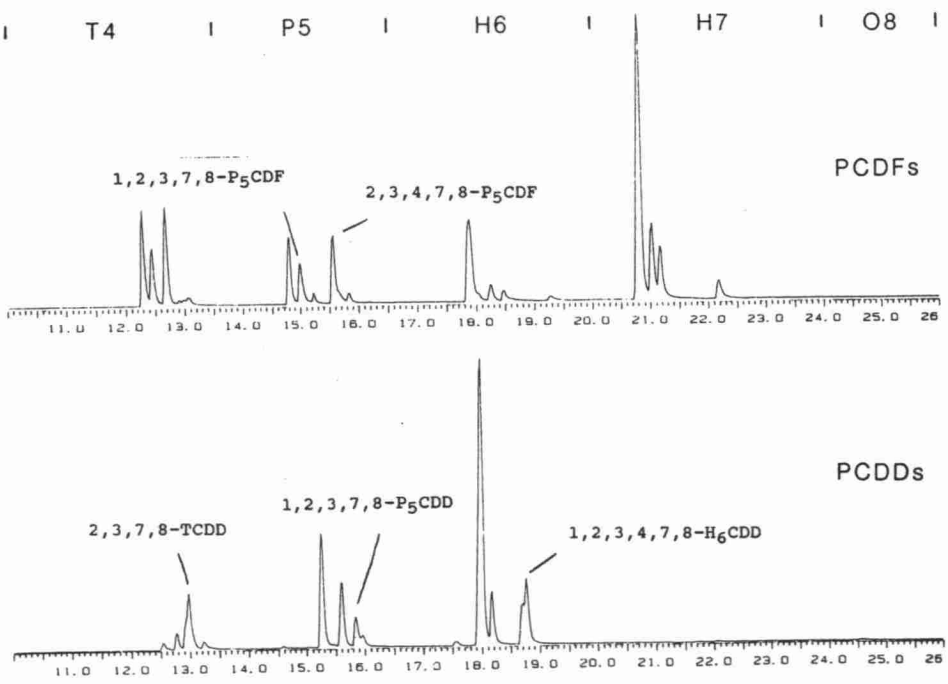


FIGURE 6: RIC of flyash fraction #3.

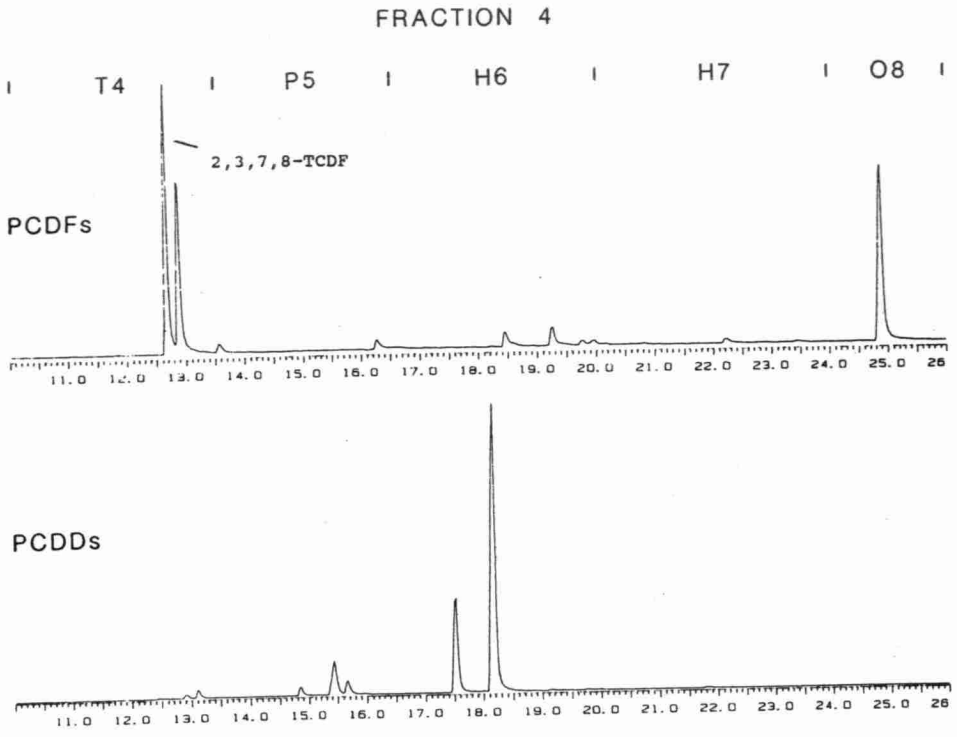
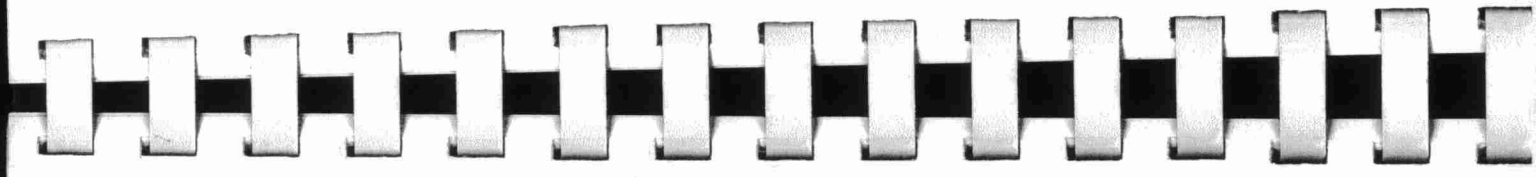


FIGURE 7: RIC of flyash fraction #4.



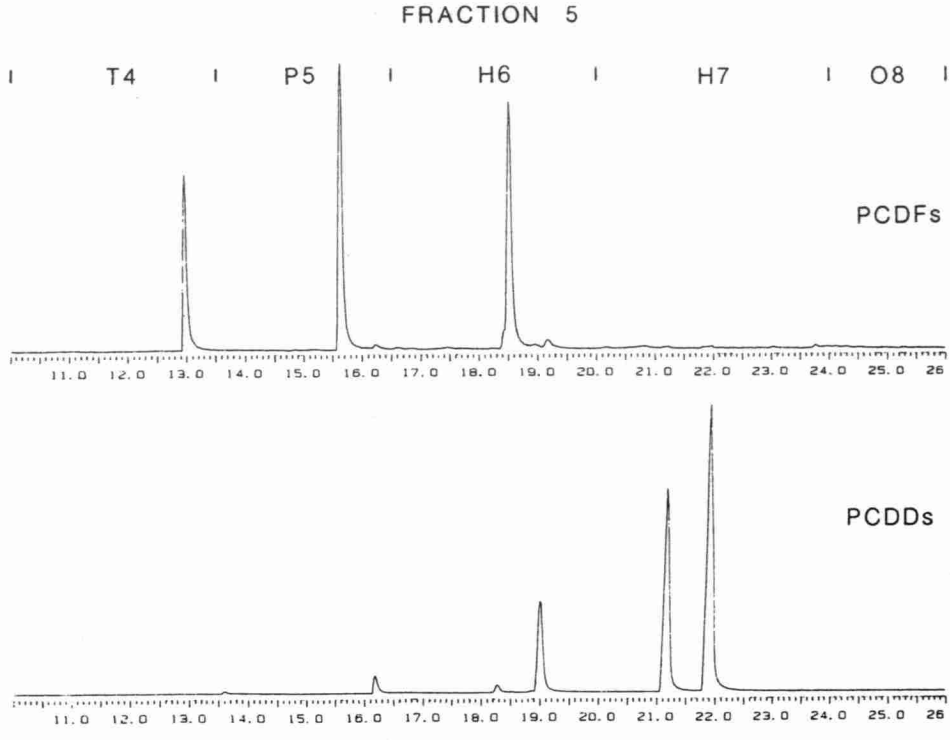


FIGURE 8: RIC of flyash fraction #5.

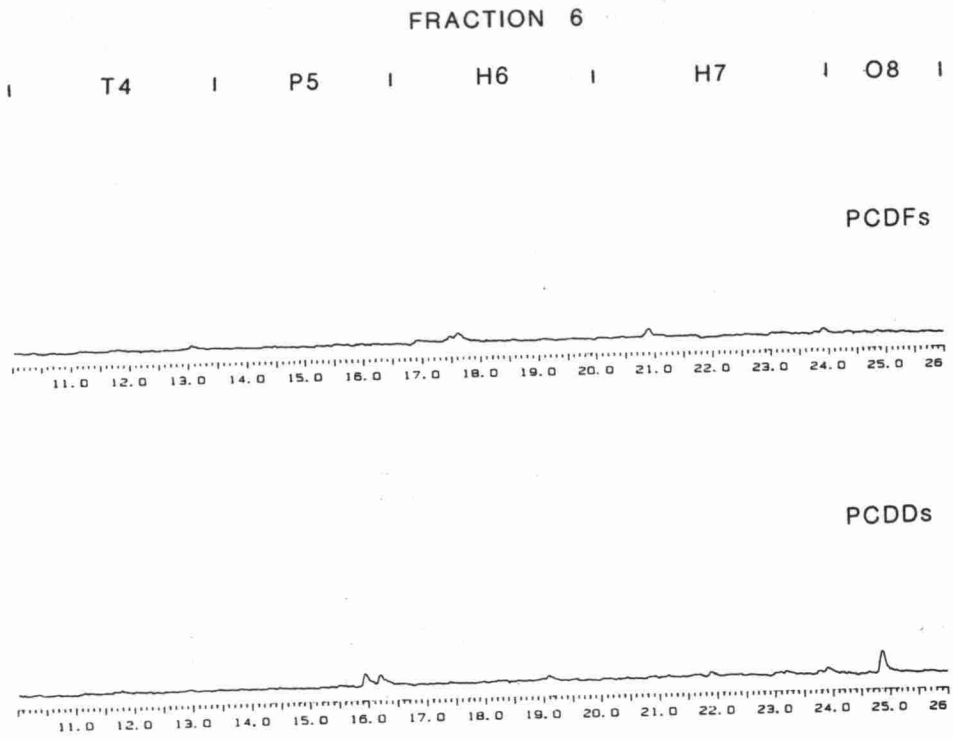


FIGURE 9: RIC of flyash fraction #6.

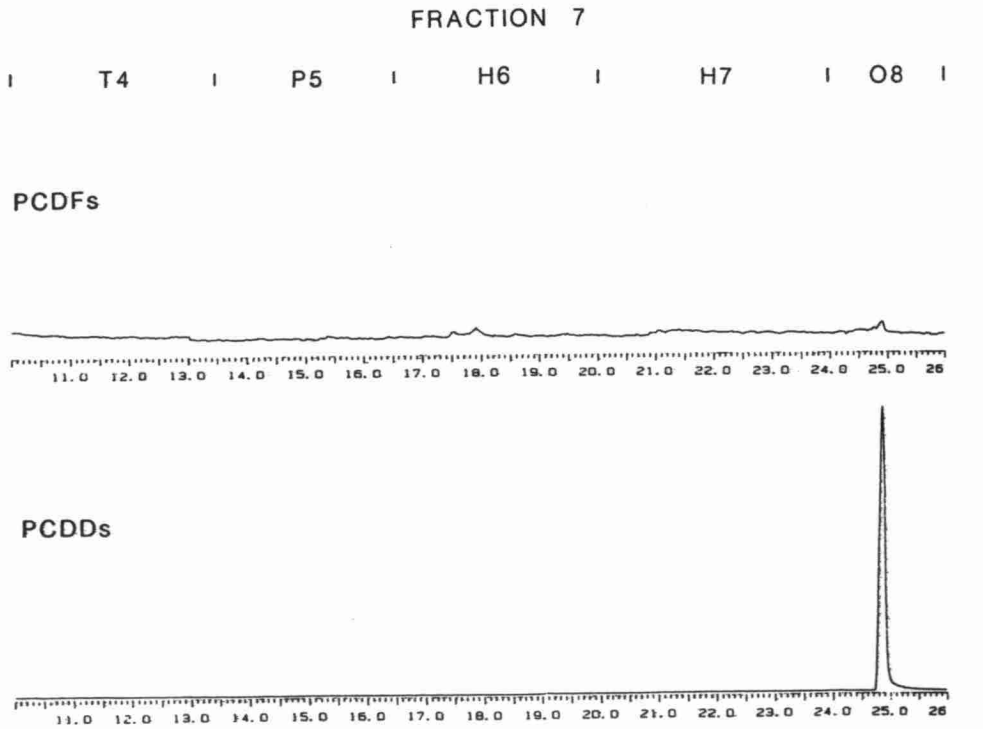


FIGURE 10: RIC of flyash fraction #7.